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(54) Title: CONTACT-KILLING NON-LEACHING ANTIMICROBIAL MATERIALS

(57) Abstract

An antimicrobial material is described which can be used to form on the surface on a substrate a non-leaching antimicrobial coating or layer which kills microorganisms on contact. The non-leaching antimicrobial coating or layer is a unique combination of an organic matrix immobilized on the surface of the substrate to having biocidal metallic materials non-leachably associated with the matrix. When a microorganism contacts the coating or layer, the biocidal metallic material is transferred to the microorganism in amounts sufficient to kill it. Methods of applying the coating or layer to a substrate also are provided.

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CONTACT-KILLING NON-LEACHING ANTIMICROBIAL MATERIALS

Field of the Invention

The present invention relates to non-leaching antimicrobial materials, specifically, the provision of antimicrobial materials capable of killing microorganisms on contact, as well as methods of manufacture and use of such materials.

Background of the Invention

The constant threat of bacterial contamination and the associated repercussions on health have made preservatives a ubiquitous part of drugs and packaged food. However, preservatives oftentimes have undesirable side effects, especially in pharmaceutical products. Growing consumer awareness about the deleterious effect of preservatives in recent years has necessitated their reduction or preferably, total elimination, without risking bacterial contamination, thus prompting the need for the development of new, cost effective packaging and storing methods that prevent bacterial contamination. The problem is acute in the pharmaceutical area, especially in the ophthalmic industry, which is presently driven by the need to address the issue of patient sensitivity toward preservatives in ocular solutions. Burnstein, N.L. et al., Trans. Ophthalmol. Soc., 104: H02 (1985); Collins, H.B. et al., Am. J. Optom. & Physiolog. Optics, 51: 215 (A89). Similar problem, exist in the food, medical device, healthcare and water purification areas.

The modality of action of all infection resistant surfaces presently known is via one of the following mechanisms: (i) dissolution of an antimicrobial component into the contacting solution, or (ii) chemically bound antimicrobials. The former is accomplished by blending an antimicrobial compound with a plastic material. The composite material is then either molded into a device or applied as a coating. The bactericidal action of such coatings depend on diffusion of the biotoxic agent into solution. Numerous examples of this type have been reported in the literature. Another variant of this type involves hydrolysis or dissolution of the matrix containing an antimicrobial compound, thereby effecting it's release into solution. High levels of preservatives are, however, released into contacting solutions in long term applications. In the latter mechanism, a bioactive compound is covalently bound either directly to the substrate surface or a

polymeric material that forms a nondissolving surface coating. The antimicrobial compounds in such coatings exhibit greatly diminished activity, unless assisted by hydrolytic breakdown of either the bound antimicrobial or the coating itself. In either case, relatively high levels of preservative has to be released into solution in order to elicit antimicrobial action.

Various products for use externally or internally with humans or animals can serve to introduce bacterial, viral, fungal or other undesirable infections. Such products include medical devices, surgical gloves and implements, catheters, implants and other medical implements. To prevent such contamination, such devices can be treated with an antimicrobial agent. Known methods of preparing an infection-resistant medical devices have been proposed in U.S. Pat. Nos. 3,566,874; 3,674,901; 3,695,921; 3,705,938; 3,987,797; 4,024,871; 4,318,947; 4,381,380; 4,539,234; 4,612,337; 3,699,956; 4,054,139; 4,592,920; 4,603,152; 4,667,143 and 5,019,096. However, such methods are complicated and unsatisfactory. Prior known antimicrobial coatings often leach material into the surrounding environment. Many are specifically designed for releasing antimicrobial agents (see, U.S. Pat. No. 5,019,096). There is a need for medical devices and other products which are able to resist microbial infection when used in the area of the body to which they are applied, which provide this resistance over the period of time, and which do not leach antimicrobial materials into the environment.

Summary of the Invention

It is an object of the invention to provide contact killing non-leaching antimicrobial materials which are capable of killing microorganisms on contact, but which do not leach significant amounts of antimicrobial materials into the surrounding environment. The antimicrobial materials may be deposited on the surface of a substrate to form a contact-killing antimicrobial coating on the surface, may be compounded with a polymer and cast into a freestanding antimicrobial object or film, or may be incorporated into a carrier to provide a bulk antimicrobial which can be applied as desired to form a contact-killing antimicrobial layer.

The antimicrobial materials of the present invention are molecularly designed to enable a complexed or matrix bound biocide to retain high activity without elution of any biocide into contacting solutions, carriers or other materials. The antimicrobial's activity stems from the sustained, cooperative biocidal action of its components. Selective transfer of one component from within the matrix directly to the microorganism upon contact is achieved via a "hand off" mechanism upon engagement and penetration of the microorganism's cell membrane. The antimicrobial material, therefore, maintains long term efficacy without releasing toxic elutables into the surrounding environment.

The antimicrobial material of the present invention comprises a combination of an organic material which is capable of forming a matrix, and a broad spectrum biocide complexed with or associated with the organic material. The biocide interacts sufficiently strongly with the organic material that the biocide does not leach or readily dissociate from the organic material. The organic material must possess two important properties: it must be capable of reversibly binding or complexing with the biocide, and must be capable of insinuating the biocide into the cell membrane of a microorganism which contacts it. The organic material preferably is substantially water-insoluble, and capable of dissolving into or adhering to the cell membrane surrounding the microorganism. Preferred organic materials are those which can be immobilized on a surface or incorporated into a carrier and which bind the biocide in such a manner as to preferentially release the biocide into a microorganism which contacts the material, but not into the surrounding environment. The biocide preferably is a low molecular weight metallic material that is toxic to microorganisms and is capable of complexing with or reversibly binding to the organic matrix

material, but which binds preferentially to cellular proteins of microorganisms. When a microorganism contacts the antimicrobial material, the organic material engages or penetrates at least the outer portion of the lipid bilayer of the microorganism's cell membrane sufficiently to permit insinuation of the biocide into the microorganism, where cell proteins or proteins in the lipid bilayer compete effectively for the biocide due to favorable binding constants. The result is a contact-killing delivery system that selectively transfers the biocide through or into the microorganism's cell membrane upon contact without elution of the biocide into solution, thereby maintaining long term efficacy. The unique mode of action of the presently described antimicrobial material offers high activity coupled with substantially low leachables.

Organic materials useful in the present invention comprise materials which are capable of: 1.) reversibly binding or complexing with a biocide, and 2.) insinuating the biocide into the cell membrane of the microorganism upon contact. A preferred class of materials are those having the aforementioned properties, and which are capable of complexing and/or binding an antimicrobial metallic material. Most preferred is the class of organic materials which can dissolve into, or adhere to, and penetrate at least the outer portion of the lipid bilayer membrane of a microorganism. For this purpose, surface active agents, such as cationic compounds, polycationic compounds, anionic compounds, polyanionic compounds, non-ionic compounds, polynonionic compounds or zwitterionic compounds are useful. Organic materials which currently are most preferred for use in the invention include cationic or polycationic materials such as biguanide compounds. In a preferred embodiment of the present invention, the organic material is a polymer capable of forming a matrix. It is understood that the term "polymer" as used herein includes any organic material comprising three or more repeating units, and includes oligomers, polymers, copolymers, terpolymers, etc.

In one aspect, the organic material can be an adduct formed by reaction of the organic material with a crosslinking agent or a chain-extending agent. Crosslinking agents which can be used in the present invention are those multifunctional organic compounds which react with the organic material to form an adduct which can be crosslinked to form a crosslinked network or matrix. Suitable crosslinking agents include, for example, multifunctional compounds containing organic groups such as isocyanates, epoxides, carboxylic acids, acid chlorides, acid anhydrides, succimidyl ether aldehydes or ketones, and further may include multifunctional compounds such

as alkyl methane sulfones, alkyl trifluoromethane sulfonates, alkyl paratoluene methane sulfones, alkyl halides and multifunctional epoxides. As used herein, the term "multifunctional" refers to compounds having at least three functional groups. Chain-extending agents which can be used in the present invention are mono-functional or difunctional organic compounds which react with the organic material to form an adduct but which are not necessarily capable of being crosslinked, and which are hydrophobic, that is, substantially water-insoluble. Suitable chain-extending agents include, for example, mono-functional or difunctional aliphatic hydrocarbons, heteroaliphatic hydrocarbons, aromatic and heteroaromatic hydrocarbons, organosilanes and perfluoro compounds. Examples of chain-extending agents include bisglycidyl ethers of bisphenol A, bisepoxides such as α,ω-bisglycidyl polyethylene glycol, poly[bisphenol A-coepichlorohydrin] glycidyl end capped and N,Ndiglycidyl-4-glycidyloxyaniline. In a preferred embodiment, the organic material comprises a biguanide compound. The biguanide compound may be a polymer comprising repeating biguanide units, such as polyhexamethylene biguanide, or be a co-polymer containing biguanide units and one or more additional organic materials. For example, the biguanide polymer may be a copolymer formed by reacting a polyepoxy compound and a biguanide compound. In a currently preferred embodiment, the organic material comprises an adduct formed by reacting polyhexamethylene biguanide with an epoxide, such as N,N-bismethylene diglycidylaniline. The resulting adduct can then be applied to a substrate and allowed to dry to form a noncrosslinked matrix or may be cured to form a crosslinked network or matrix.

The biocide can be any antimicrobial material which is capable of non-leachably binding or complexing with the organic material, but which, when placed in contact with a microorganism, preferentially transfers to proteins in the microorganism. For this purpose, metallic materials which bind to cellular proteins of microorganisms and are toxic to the microorganisms are preferred. The metallic material can be a metal, metal oxide, metal salt, metal complex, metal alloy or mixture thereof. Examples of such metals include, e.g., silver, zinc, cadmium, lead, mercury, antimony, gold, aluminum, copper, platinum and palladium, their salts, oxides, complexes, and alloys, and mixtures thereof. The appropriate metallic material is chosen based upon the use to which the device is to be put. The currently preferred metallic materials are silver salts. In a currently preferred embodiment, a silver halide is used, most preferably, silver iodide.

In one aspect, the antimicrobial material comprises a complex of a polycationic ligand compound and a metallic material. The polycationic compound and the metallic material form a stable, isolatable coordination complex having antimicrobial properties. In a preferred embodiment, the polycationic compound is a polymer. In another preferred aspect, the polycationic compound itself has antimicrobial activity. In a currently preferred embodiment, the polycationic compound is polyhexamethylene biguanide and the metal is silver, most preferably, silver iodide. The complex preferably is in dry form, such as a powder, comprising fine particles of the complex.

The invention further comprises liquid compositions for forming a contact killing, non-leaching antimicrobial layer or coating on a surface. In one embodiment, the composition is a two-part composition comprising a first solution, dispersion or suspension of an organic material, and a second solution, dispersion or suspension of a biocide. If a crosslinked coating or film is desired, the first solution, dispersion or suspension also will contain the crosslinking agent. As a first step, the crosslinking agent and the organic material may be reacted to form a non-crosslinked adduct. To form a contact-killing nonleaching coating or layer on a substrate, the first composition is applied to the substrate under conditions sufficient to immobilize the organic material on the substrate, forming a matrix. If a crosslinking agent is present, the matrix can be cured to induce crosslinking. The matrix then is exposed to the solution of the biocidal material under conditions sufficient induce the biocide to become non-leachably attached to, complexed with or associated with the matrix.

In another embodiment, the liquid composition is a one part composition comprising a solution, dispersion or suspension of the organic material, the biocide, and optionally, the crosslinker. To form the contact-killing coating on a substrate, this composition is applied to the substrate under conditions sufficient to immobilize the organic material on the substrate, forming a matrix in which the biocide is non-leachably attached to or associated with the matrix.

The dry powder, and the two part or one part liquid compositions also may be used to make freestanding antimicrobial films, microbeads or other solid shapes as described in more detail below. As used herein, the term "freestanding" means not attached to a substrate.

The invention further provides methods for making the compositions of the present invention, and applying them to various substrates to form antimicrobial coatings or layers on the substrates, or compounding them with a carrier.

Generally, the compositions are made by combining the organic polycationic material with the metallic biocide under conditions appropriate to form the complex. The conditions may vary depending upon the polycationic materials and metallic biocide selected. In one embodiment, the complex is formed by contacting a liquid solution of the polycationic material with a liquid solution of the biocidal metallic material, resulting in formation of the complex as a precipitate from the solution. The precipitate then can be dried and ground to form a powder.

To make the dry or powder compositions of the invention, the organic polycationic material and the metallic biocide first may be combined in a liquid carrier to form a solution, dispersion or suspension of the complex, which then may be dried to evaporate the liquid. The drying step may be performed in any suitable manner to obtain the desired product, including spray drying, air drying, heating, etc. In one embodiment, a powder form of the complex can be prepared by combining a liquid solution of the organic material and the biocide to form a solution, dispersion or suspension of the organic material:biocide complex. The solution, dispersion or suspension then is cast as a film onto a non-adherent substrate and dried to form a film. The film then is detached from the non-adherent substrate and ground to a powder. The term "non-adherent substrate" means a substrate to which the coating or film formed from the complex will not bond, and from which it can be removed intact. In another embodiment, a complex is formed between a crosslinked form of the organic material and the biocidal metallic material. In this embodiment, the organic material is reacted with a crosslinking agent to form an adduct. The adduct then is cured to induce crosslinking. The resulting crosslinked material then is contacted with the biocidal metallic material under conditions sufficient to form the complex.

In a preferred embodiment, the organic material is polyhexamethylene biguanide (PHMB) or an adduct formed by the reaction of PHMB with an epoxy functional compound, preferably N,N-methylenebisdiglycidylaniline (MBDGA). The adduct is formed by reacting PHMB and BMDGA by heating a mixture of the two components at a temperature of from about 90 to about 95°C for about 15 minutes. The PHMB or PHMB adduct then is combined with the metallic

biocide, preferably a silver salt, thereby forming a precipitate containing a complex of PHMB:MBGDA:AgI. The currently preferred silver salt is silver iodide. The resulting precipitate then is dried and can be ground to form a fine powder of the complex. Techniques for making dry powders of complexes based on organic materials and/or biocides other than PHMB and silver can be accomplished using reaction conditions and drying protocols known and available to those skilled in the art.

To make the liquid compositions of the invention, a solution, dispersion or suspension of an organic material can be made or, if appropriate, as it available from the manufacturer. For example, polycationic polymers may be available in resin form (i.e., in a liquid carrier) which may be suitable for use as is, or with a slight adjustment, e.g., in the solid content. Polymer resins also may be blended with other resins or compounds; or the polymers may be reacted with or derivatized with other polymers or compounds to form copolymers, functionalized polymers or adducts. The binding and/or reaction conditions will depend upon the materials selected.

In one embodiment, a liquid solution of a polycationic organic material, such as PHMB, can be used as the organic material. PHMB can be used as is, or can be reacted with another organic polymer or compound to form a copolymer, adduct or functionalized derivative. Protocols for formation of copolymers, adducts and derivatives are well known in the chemical art. In a currently preferred embodiment, described above in connection with the procedure for forming the dry composition, PHMB first is reacted with an epoxy functional compound to form an adduct. The resulting liquid solution containing the adduct can be applied as is and subsequently impregnated with the biocide as described below, or can be combined with the biocide to form a solution, dispersion or suspension of a PHMB adduct: biocide complex. In the currently preferred embodiment, PHMB is reacted with BMDGA to form the adduct, and the adduct is combined with a silver salt, preferably silver iodide, to form a PHMB:MBDGA:AgI complex. As will be readily apparent to those skilled in the art, these techniques can be used to make complexes of organic materials and biocides based on materials other than PHMB, MBGDA and AgI provided that the materials have the required functional characteristics, that is, the ability to form a complex which has antimicrobial properties and will not leach or release the biocide into a liquid or other substance in contact with the complex, but which will preferentially transfer the biocide to a microorganism in contact with the complex.

The method for applying the liquid compositions to form an antimicrobial coating generally comprises providing a solution, dispersion or suspension the organic material, and, if a non-crosslinked material is desired, coating the solution, dispersion or suspension of the organic material onto the substrate, and drying the coating, thereby forming a matrix.

If a crosslinked coating is desired, the organic material first is combined with a crosslinking agent. Typically, both the organic material and the crosslinker will be in liquid form (e.g., in a solution, dispersion or suspension), and the two solutions are combined, forming a liquid mixture. The liquid may be an organic solvent, an aqueous liquid or a mixture of an organic solvent and an aqueous liquid. The organic material and the crosslinking agent then are reacted to form an adduct. The resulting adduct can be stored for later use, if desired, or can be immediately applied to a substrate.

Liquid compositions containing the organic material (with or without the added crosslinker) can be applied to the substrate of choice by any suitable means for applying a liquid coating, including, for example, spraying, brushing, dipping, calendering, rod or curtain coating. The method selected to apply the composition to the substrate will depend on several factors, including the coating thickness desired and the nature and configuration of the substrate. If necessary, the surface to be coated can be cleaned or treated before the polymer solution is applied. The resulting coating is dried to form the matrix, and, if crosslinking is desired, subjected to crosslinking conditions, forming a crosslinked network. Crosslinking conditions may include thermal curing, ultraviolet curing, chemical curing or other curing methods. The matrix then is contacted with a solution of the biocide under conditions sufficient to deposit the biocidal material into the matrix such that the biocidal material becomes non-leachably associated with or attached to the matrix.

Another embodiment of the method of making the coatings of the present invention comprises first combining the organic material and the biocide, then applying the mixture to the substrate to form the matrix as described above. If a crosslinked coating is desired, the organic material and crosslinking agent are reacted to form an adduct as described above, then the adduct is combined with the biocide. The resulting adduct/biocide mixture can be stored for later use, or can be immediately applied to a substrate and cured as described above to induce crosslinking,

thereby forming the polymeric network having the biocide non-leachably associated therewith or attached thereto.

In the methods of the invention described above, the amounts and/or concentrations of the materials used will depend upon the nature and stoichiometry of the materials used, and the end product desired. In the currently preferred embodiments, the concentration of the solution, dispersion or suspension of the organic material, or the organic adduct resin formed by the reaction of the polymer and crosslinker, typically is in the range of from about 0.5 to about 20% by weight. Typically, a polymer:crosslinker ratio in the range of from about 1:1 to about 3:1 (weight percent) will form crosslinked networks which will non-leachably retain the biocide and preferentially transfer the biocide to the microorganism upon contact as described herein. Solutions of the biocidal material typically comprising from about 0.005 to about 0.5 % by weight can be used to impregnate the matrix with biocide.

In another embodiment of the present method, a freestanding antimicrobial material may be formed using the present antimicrobial material. In this embodiment, using the two-part compositions described above, a solution, suspension or dispersion of the organic material is cast on a non-adherent substrate and dried to form a film. If a crosslinked material is desired, the organic material and crosslinker first are combined and reacted to form an adduct as described above, and a solution, suspension or dispersion of the adduct is cast to form the film. The film is cured to induce crosslinking, as described above. The film then is contacted with a solution, dispersion or suspension of the biocidal material to deposit the biocidal material within the matrix of organic material. The film then is detached from the substrate and used as desired. Alternatively, freestanding crosslinked or non-crosslinked films can be cast using the one part liquid compositions described above. Freestanding antimicrobial materials also may be prepared using the antimicrobial materials of the present invention in other physical forms besides films, including as microbeads or solid shapes, for example, which can be prepared by compounding the antimicrobial powder with a suitable carrier, then casting or molding the object using well known techniques.

In another embodiment, an antimicrobial powder may be formed by casting a freestanding film, as described above, then grinding the film to a powder. The powder also may be formed by

precipitating from solution the complex between the organic material and the biocidal metallic material, drying the precipitate and grinding it to form the powder. The powder has similar contact-killing antimicrobial properties to the films and coatings described above. The antimicrobial powder can be incorporated into a carrier, such as a gel, cream or liquid, and applied to a surface to form an antimicrobial layer. For example, a formulation comprising the antimicrobial powder dispersed in a pharmaceutically acceptable carrier can be used as a topical antiseptic and be applied to a wound.

In a preferred embodiment, the antimicrobial materials of the present invention are used to form a contact-killing surface on a substrate. To provide the contact-killing surface on the substrate, the organic compound may be attached to and/or immobilized on the substrate by any appropriate method, including covalent bonding, ionic interaction, coulombic interaction, hydrogen bonding, crosslinking (e.g., as crosslinked (cured) networks) or as interpenetrating networks, for example.

In a currently preferred embodiment, the organic matrix is formed by first reacting polyhexamethylenebiguanide with N,N-bismethylene diglycidylaniline to form an adduct. Stable coating solutions of the resulting adduct have been obtained in both absolute ethanol and in aqueous ethanol. The adduct can be applied on a substrate surface either by dip-coating, brushing or spraying. Once applied to the substrate, the coating is dried to thereby form a matrix. The coating can be cured (e.g., by heating) to induce crosslinking, thereby forming a crosslinked polymeric network on the substrate. The resulting coating is optically clear, resistant to most solvents and to temperature changes, and does not delaminate, flake or crack. The coating typically is about ten microns or less in thickness, although the thickness of the coating may be varied by well- known techniques, such as increasing the solids content of the resin. A broad spectrum metallic antimicrobial, preferably a silver compound, then is introduced into the polymeric network such that it is entrapped as submicron particles, and complexes with the functional groups on the polymer. Alternatively, the metallic material is combined with the liquid containing the polymer prior to applying it to the substrate. In the currently preferred embodiment, the broad spectrum antimicrobial is a silver halide, preferably silver iodide.

The antimicrobial materials of the present invention are unique in the following respects:

i) The unique nature of the antimicrobial material utilizes a cooperative effect of it's components. This results in high biocidal activity, while maintaining almost no significant leachables into solutions it is in contact with.

ii) The mechanism of action is essentially a surface mediated one, whereby organisms succumb only upon contact with the material due to the non-leaching property associated with it.

iii) The ability of such surfaces to remain completely inert in solution in the absence of microorganism contamination.

iv) The ability of such surfaces to remain viable over multiple organism challenges with no decrease in their bioactivity.

v) The utilization of such biocidal materials on an interior or exterior surface of a device, thereby eliminating the possibility of microbial colonization on the surface.

vi) User friendliness and cost effectiveness of the coating for all types of applications.

vii) Adaptability to existing manufacturing technology, thereby enabling large scale manufacture with minimal cost.

The above and other objects, features and advantages of the present invention will be better understood from the following specification when read in conjunction with the accompanying drawings.

Brief Description of the Drawings

Figure 1A is a schematic graphic illustration of the matrix/biocide complex of the present invention, before contact of the coating with microorganisms.

Figure 1B is a schematic graphic illustration of the contact-killing ability of the matrix/biocide complex of the present invention during contact of the coating with microorganisms.

Figures 2A-D are a schematic graphic illustration of a preferred method for applying the matrix/biocide complex of the present invention to a substrate:

Figure 2A shows the matrix immobilized on the substrate, with chains of the organic material forming arms or tentacles that protrude into the surrounding environment;

Figure 2B shows the immobilized matrix impregnated with a biocidal compound, with reservoirs of the biocide deposited within the matrix and molecules of the biocidal compound attached to the tentacles;

Figure 2C shows a microorganism in contact with the matrix/biocide complex wherein the polymer chains engage and dissolve into the microorganism cell membrane;

Figure 2D shows penetration of the cell membrane and transfer of the biocide from the network into the microorganism, causing cell death.

Figure 3 is a graph illustrating the bioactivity of a preferred coating of the present invention, a matrix formed from crosslinked PHMB complexed with silver salts, treated as a function of surface area to volume against *Pseudomonas aeruginosa* microorganisms in phosphate buffered-saline at 30°C.

Detailed Description

The antimicrobial materials of the present invention can be combined with a variety of carriers to form bulk antimicrobial compositions, or can be coated onto a variety of substrates to form an antimicrobial coating. Both the bulk antimicrobials and the coatings are non-leaching and contact-killing. That is, they do not leach significant amounts of antimicrobial components into the surrounding environment, and will kill most microorganisms which come into contact with them.

The term "microorganism" as used herein includes bacteria, blue-green algae, fungi, yeast, mycoplasmids, protozoa and algae.

The term "biocidal" as used herein means the killing of microorganisms, or inhibiting the growth of microorganisms, which can be reversible under certain conditions.

As used herein, the terms "non-leachable" or "substantially non-leachable" means that none or very minute amounts (e.g., below a certain threshold) of the organic and/or biocidal material dissolves into a liquid environment. Preferably, this threshold is no higher than 1 part per million (ppm), and more preferably is lower than 100 parts per billion (ppb).

Organic materials useful in the present invention comprise materials which are capable of: 1.) reversibly binding or complexing with the biocide, and 2.) insinuating the biocide into the cell membrane of the microorganism. A preferred class of materials are those having the aforementioned properties, which are capable of being immobilized on a surface and which preferentially bind a biocidal metallic material in such a manner so as to permit release of the metallic biocide to the microorganism but not to the contacting environment. Most preferred is the class of organic materials which can dissolve into, adhere to, disrupt or penetrate the lipid bilayer membrane of a microorganism. For this purpose, surface active agents, such as cationic compounds, polycationic compounds, anionic compounds, polyanionic compounds, non-ionic compounds, polyanionic compounds or zwitterionic compounds may be used. Organic materials which currently are most preferred for use in the invention include cationic or polycationic compounds such as biguanide compounds.

Preferred cationic materials include benzalkoniumchloride derivatives, a-4-[1-tris(2-hydroxyethyl) ammonium-2-butenyl] poly[l-dimethylammonium-2-butenyl]-ω -tris(2-hydroxyethyl) ammonium chloride, and biguanides of the general formula:

$$Y_1\text{-}[\text{-NH-C(=NH)-NH-C(=NH)-NH-X-}]_n\text{-}Y_2$$

or their water soluble salts, where X is any aliphatic, cycloaliphatic, aromatic, substituted aliphatic, substituted aromatic, heteroaliphatic, heterocyclic, or heteroaromatic compound, or a mixture of any of these, and Y_1 and Y_2 are any aliphatic, cycloaliphatic, aromatic, substituted aliphatic, substituted aromatic, heteroaliphatic, heterocyclic, or heteroaromatic compound, or a mixture of any of these, and where n is an integer equal to or greater than 1. Preferred compounds include, e.g., chlorhexidine (available from Aldrich Chemical Co., Milwaukee, WI) or polyhexamethylene biguanide (available from Zeneca Biocides, Inc. of Wilmington, DE). The above-mentioned organic materials may be modified to include a thiol group in their structure so as to allow for the bonding of the compound to a metallic substrate, or may be derivatized with other functional groups to permit direct immobilization on a non-metallic substrate. For example, the above-mentioned organic materials may be suitably functionalized to incorporate groups such as hydroxy, amine, halogen, epoxy, alkyl or alkoxy silyl functionalities to enable direct immobilization to a surface.

In a preferred embodiment of the present invention, the organic material comprises a polycationic material which is crosslinked to form the matrix. Crosslinking agents which can be used in the present invention are those which react with the polycationic material to form an adduct which then can be reacted to form a crosslinked network or matrix. Suitable crosslinking agents include, for example, compounds containing organic multifunctional groups such as isocyanates, epoxides, carboxylic acids, acid chlorides, acid anhydrides, succimidyl ether aldehydes and ketones, and organic compounds such as alkyl methane sulfones, alkyl trifluoromethane sulfonates, alkyl paratoluene methane sulfones, alkyl halides and organic multifunctional epoxides. In a currently preferred embodiment, a polyhexamethylene biguanide polymer is reacted with an epoxide, such as N,N-methylene bisdiglycidylaniline, which then is cured to form a crosslinked network.

The biocidal material can be any antimicrobial material which is capable of non-leachably binding to or complexing with the organic matrix, but which, when placed in contact with the microorganism, preferentially transfers to the microorganism. For this purpose, metallic materials which are toxic to microorganisms are preferred. The metallic material can be a metal, metal oxide, metal salt, metal complex, metal alloy or mixture thereof. Examples of such metals include, e.g., silver, zinc, cadmium, lead, mercury, antimony, gold, aluminum, copper, platinum and palladium, their oxides, salts, complexes and alloys, and mixtures of these. The appropriate metallic material is chosen based upon the use to which the device is to be put. The currently preferred metallic materials are silver compounds.

The biocidal material can be introduced into the matrix either contemporaneously with or after application of the organic material to a surface.

The invention also provides a substrate in which the surface is at least partially coated with additional organic materials, and/or biocidal materials, or both. Examples of organic and biocidal materials that can be used are discussed above. The use of a combination of at least two different organic and biocidal materials can enhance the antimicrobial properties of the coating. Different types of microorganisms can exhibit different degrees of sensitivity to different organic and biocidal materials. In addition, the use of two or more different organic and biocidal materials can significantly reduce the problem of selection for microorganisms having resistance to the organic and biocidal materials in the coating that can occur when only one is used.

The amount and/or type of the antimicrobial coating which is used in a particular application will vary depending on several factors, including the type and amount of contamination which is likely to occur, and the size of the antimicrobial surface. The amount of antimicrobial used will be a minimum amount necessary to maintain the sterility of the liquid. As stated above, this amount will vary depending upon various considerations.

In a preferred embodiment, the organic material, whether crosslinked or non-crosslinked, forms an insoluble, non-leachable matrix having a unique configuration: some of the organic material protrudes into the surrounding environment, that is, "arms" or "tentacles" of the organic material project away from the matrix and into the surrounding environment. This phenomenon

can be understood by referring to Figures 1 and 2, which are schematic graphic illustrations of a preferred coating of the present invention in which the organic material is a crosslinked biguanide polymer and the biocidal material is a silver halide salt, preferably silver iodide. Figures 1A and 1B and Figures 2A-D show the polymer matrix having tentacles projecting into the ambient environment, and the silver salt deposited in reservoirs and on the tentacles. Without wishing to be bound by theory, it is believed that when a microorganism contacts the coating, the biguanide polymer tentacles dissolve into the lipid bilayer surrounding the microorganism, thereby introducing silver molecules into the interior of the microorganism or to proteins within the cell membrane. The silver salt has a greater affinity for certain proteins in the microorganism than for the polymer, and therefore complexes with the cellular proteins and is transferred into the microorganism, thereby killing it. Specifically, it is thought that the silver forms complexes with the sulfhydryl and amino groups of the cellular proteins.

In this embodiment, the silver salt is attached to or impregnated into the matrix and on the tentacles of the polymer such that the silver is substantially non-leachable. Again, not wishing to be bound by theory, it is believed that the silver salt forms complexes with functional groups in the polymer, and that the complexed silver resists leaching into ambient liquids or other materials (e.g., creams or gels) in contact with the coated surface. However, when the coating becomes exposed to cellular proteins, the silver preferentially complexes with the proteins.

In a currently preferred embodiment, the polymeric material is polyhexamethylene biguanide (PHMB), the crosslinking agent is N,N-methylenebisdiglycidylaniline (MBDGA), and the silver salt is a silver halide, most preferably, silver iodide. In this embodiment, the liquid composition is made by combining a solution of polyhexamethylene biguanide with a solution of the crosslinking agent, and reacting the mixture under conditions sufficient to form a non-crosslinked PHMB-MBDGA adduct. The ratio of PHMB to MBDGA preferably is in the range of from about 1:1 to 3:1 by weight. The PHMB-MBDGA mixture is heated to about 95°C for about 2 hours in a closed reactor to form the adduct. The concentration of the resulting adduct resin preferably is in the range of from about 0.5 to about 20% by weight. To form a contact-killing antimicrobial coating, the adduct resin solution is coated onto the desired substrate, and heated to a temperature sufficient to induce crosslinking between the adducts, thereby forming a crosslinked network or matrix. Temperatures sufficient for crosslinking typically are in the range

of from about 70°C to about 200°C. The resulting crosslinked network is then saturated with silver by immersing the coating for about two minutes in a silver iodide/potassium iodide solution. Silver solutions having a concentration of from about 0.005 to about 0.5% can be used for this step. The silver iodide forms reservoirs in the matrix, and becomes attached to the tentacles. Silver iodide has sufficient affinity for the PHMB polymer that it forms an insoluble complex that will not leach into ambient solutions or other materials in contact with the material, even at elevated temperatures. However, when a microorganism contacts the coating, the tentacles disrupt the microorganism's lipid bilayer membrane, thereby introducing the silver iodide into the microorganism. Silver iodide has greater affinity for certain proteins within the microorganism than for the PHMB-MBDGA matrix, and forms complexes with these proteins, that is, the silver is preferentially transferred from the coating to the microorganism. The silver accumulates to toxic levels in the microorganism and kills it. The silver iodide reservoirs within the matrix replenish the silver iodide on the tentacles lost to the microorganism by reestablishing the equilibrium for formation of the complex (AgI + PHMB $\rightleftharpoons$ [PHMBAgI]).

This invention also includes the coated substrates, freestanding films, powders and articles made in accordance with the above methods.

The present invention provides stable, adherent coatings or layers using the present coating formulations on a wide range of materials, including those commonly used in membranes and in medical device manufacture. Antimicrobial coatings according to the present invention can be applied, for example, to woods, metals, paper, synthetic polymers (plastics), natural and synthetic fibers, natural and synthetic rubbers, cloth, glasses, and ceramics. Examples of synthetic polymers include elastically deformable polymers which may be thermosetting or thermoplastic such as, for example, polypropylene, polyethylene, polyvinylchloride, polyethylene terephthalate, polyurethane, polyesters, rubbers such as polyisoprene or polybutadiene, polytetrafluoroethylene, polysulfone and polyethersulfone polymers or copolymers. The substrate can be a deformable metallic or plastic medicament container, such as a toothpaste tube, where the container may remain deformed after each dose is dispensed. Other polymeric materials, including polymeric materials which are used for the preparation of membranes or filter papers, also can serve as substrates. Examples of organic polymeric materials include polyamide (e.g., nylon), polycarbonate, polyacrylate, polyvinylidene fluoride, cellulosics (e.g., cellulose), and Teflon®.

The substrate can be either hydrophilic or hydrophobic. With the exception of silicone and Teflon®, which may require prior surface activation with techniques such as plasma, chemical oxidation or metallic sensitization, e.g., a primer, no surface activation is necessary. Inorganic materials to which the present coatings can be applied include glass fiber materials, ceramics such as alumina or silica, and metals. Sintered glass and sintered ceramic substrates also can be used.

The coating or layer may be applied directly to most surfaces without prior surface modification. Studies simulating a year of contact between the coating and aqueous solutions at ambient temperature resulted in less than 100 ppb of any active ingredient in the solution. The extract solutions themselves (solutions which have been in contact with the coating) show no antimicrobial or mammalian cell toxicity. The coated surface remains fully inert and bio-active after exposure to various physical and chemical stresses including: low temperature, ethanol, boiling water, prolonged exposure to varying pH solutions and solutions of high ionic strength, as well as sterilization by conventional methods (e.g., wet autoclave, ethylene oxide, γ-irradiation, ethanol).

Surface coatings, freestanding films and formulations containing the antimicrobial powder according to the present invention exhibit antimicrobial activity against both gram positive and gram negative bacteria and yeast, and are resistant to fungal growth. Treated surfaces completely kill organisms at challenge levels of 10^6 - 10^8 CFU/mL within 8 to 20 hours at 30°C, depending on organism type. Tables 1 and 2 (in Example 4) list the bioactivity of coated surfaces towards different challenge organisms. The coating renders surfaces biofilm resistant, which coupled with its chemical inertness, makes it particularly suited for many device applications.

The antimicrobial materials of the present invention have been successfully applied on the surface of microporous membranes, including within the pores as evidenced by SEM-EDX. Stable, uniform coatings have been obtained on a variety of membrane materials with almost no reduction in their flow property. Coated microporous membranes kill micro organisms upon contact and are resistant to the phenomenon of "bacterial grow-through" which occurs even in sterilizing 0.2 μM pore size membranes in long term contact applications. Such membranes are, therefore, well suited for incorporation in devices used in long term filtration applications such as

multi-dose dispensers for preservative free formulations, water purification systems and in any application where it is desirable to use barrier properties of a membrane for more than a day.

The mechanism of action is one wherein the antimicrobial materials are activated only upon contact with the microorganism. Once the microorganism accumulates a toxic amount of silver, it succumbs and detaches from the surface. The coating or other treated surface, therefore, remains active only as long as viable organisms contact it, and reverts to being inert in their absence. This unique property whereby the biological activity is triggered by bacterial cell contact enables the coating to function "intelligently." For such a contact mechanism to be effective, the rate of kill is expected to vary as a function of the ratio of total surface area of coated substrate to the volume of the bacterial suspension in contact with it (S/V ratio) at constant temperature. As shown in the Examples, time to kill experiments were performed on coated polyethylene tubes of varying inner diameter that were inoculated with predetermined volumes of a suspension containing 10^6 CFU/mL of *Pseudomonas aeruginosa* in phosphate buffered saline (PBS). The decrease in organism concentration was measured as a function of time at constant temperature over 20 hours. Experimental results are summarized in Figure 3. There is no substantial difference in kill rate for S/V ratios ranging from 2.5 to 5 cm^{-1}; similar results were obtained for a ratio of 1.5. For the largest diameter tubes tested (S/V = 0.5), however, viable organisms were detected at low levels, which can be attributed to a decrease in probability of organisms contacting the surface with increasing volumes. No toxic components were found in organism-free solutions in contact with coated tubes under identical conditions when tested both chemically and biologically, which supports the proposed contact mechanism for cell death. Such a distinction would not be evident if sterilization were to occur via either controlled dissolution or diffusive elution of the coating components into solution; in either case, high levels of active components would be present in solution.

The antimicrobial materials of the present invention can be used to form contact-killing coatings or layers on a variety of substrates. As shown in the Examples, the material forms a non-leaching contact-killing surface on materials which are used in medical devices which are implanted, inserted or in intimate contact with a patient, such as catheters, urological devices, blood collection and transfer devices, tracheotomy devices, valves, stents, intraocular lenses, and on personal or health care products which topically contact the patient or other user such as

toothbrushes, contact lens cases and dental equipment. The antimicrobial materials of the present invention in powder form can be dispersed or dissolved in a carrier and used as a topical antiseptic, wound dressing or topical disinfectant. Such carriers may include creams, gels, lotions, soaps or other topically applicable materials. The materials can be used on medical devices and healthcare devices and products, consumer products, baby care products, personal hygiene products, household products, bathroom accessories including shower enclosures, toilet seats, sinks and countertops, kitchen surfaces, food preparation surfaces and packaging, water storage, treatment and delivery systems, biosensitive systems and laboratory and scientific equipment.

For example, contact lens cases are a proven contributor to the spread of ocular pathogens and disease. A lens case coated with a coating of the present invention has been shown *in vitro* to kill all clinically relevant pathological strains of micro-organism without leaching toxic chemicals into the contact lens solution (see Examples 2 and 4). Once a bio-film has formed on an untreated contact lens case, it is resists virtually all types of disinfection products currently available for contact lens care. Thus, the bio-film serves as a reservoir for bacteria that re-contaminate the lens each time it is stored in the case. The treated lens case is compatible with all disinfecting solutions tested to date. Use of the coating permits sterilization of the lenses using ordinary saline as the soaking solution.

The present antimicrobial materials can be used to coat ordinary nylon bristle toothbrushes (see Example 2). The treated toothbrush kills the pathogens commonly found in the human mouth and on bathroom surfaces, while untreated toothbrushes foster their growth. It is believed that toothbrushes are partly responsible for the spread of oral and dental disease. *In vitro* and *in vivo* test programs examined the types, number and kill-rates for the organisms commonly found in the mouth. The tests indicated that the treated toothbrush eliminated virtually all of these pathogens over a 12 hour period. The inert coating does not elute from the brush and therefore has no taste and poses no risk to the consumer. The present materials also can be used to provide an antimicrobial layer or to kill microbes on dental instruments, dental floss, and other devices for use in the mouth.

Bio-film formation is a major problem in many water container, water filtration, and water delivery applications. Once a bio-film is formed, it typically resists further treatment and acts as a

constant source of microbial contamination. Prevention of bio-film formation is key to the maintenance of high quality water systems. The present materials can be used to prevent bio-film formation on many water treatment products. For example, water containers and water purification systems used in camping, residential, commercial and military applications, which need to be periodically emptied, disinfected and rinsed. Treatment with the present antimicrobial materials would eliminate the costs and hazards associated with this process, as well as the risks associated with improper maintenance of these water storage systems.

The present antimicrobial materials also are useful in point-of-use water purification filters, which trap bacteria and nutrients commonly found in all water systems. The bio-films formed in these filters often shed bacteria into the water stream in quantities exceeding the standard safety limits. Treated filters would offer longer service life and significantly reduce the potential for bio-hazard.

Surfaces in medical offices, such as treatment tables, or consoles in a typical dental office have proven to be a major source of bacterial contamination, posing potential health risks to the patient and staff. Although water supplies are routinely treated to reduce bio-contamination, water standing in the lines in the dental console can promote the formation of bio-films. Coating or treating these surfaces with the antimicrobial materials of the present invention can prevent bio-film formation on these substrates.

The present antimicrobial materials have been tested against the bacteria most commonly found in water. Treated tubing withstood repeated attempts to induce bio-film formation at very high challenge levels, while untreated control tubing developed extensive bio-film (see Examples 5 and 11). The treated tubing showed no traces of chemical elution into the water.

The present materials also can be applied to woven and non-woven fabrics used in hospitals and on healthcare supplies ranging from face masks to bed sheets. The materials can be applied in a spray or wipe form which can be applied to surfaces in order to make them antimicrobial.

Long term indwelling catheters pose a risk of infections (2%-9%) which increases patient discomfort, the risk of systemic infections and the length of the patient's hospital stay. Catheters treated with the present antimicrobial materials can reduce the presence of infection causing bacteria. The materials also can be used on urinary catheters, implants and inserts designed to deal with incontinence suffer from increased risk of infection. Coatings made with the present materials have been demonstrated to kill microorganism in human urine.

The antimicrobial materials of the present invention can be used to treat standard biological plastic laboratoryware for applications which require low microbiological contamination, e.g. cell culture lab ware.

EXAMPLES

Example 1

Preparation of PHMB-BMDGA Solutions

Polyhexamethylene biguanide (PHMB) (available as a 20% aqueous solution from Zeneca Biocides, Wilmington, DE) was distilled to remove the water, and the PHMB was redissolved in absolute ethanol to give a 20% by weight solution. This solution was used to prepare the resins outlined below.

(a) 312 mL of the 20% PHMB solution in ethanol was further diluted with 600 ml of ethanol. This solution was added to a solution of N,N-methylene bisdiglycidylaniline) (MBDGA) (Aldrich Chemical Company, Milwaukee, WI) containing 37.60 grams of MBDGA dissolved in 119.9 ml of acetonitrile and 280.1 ml of ethanol. The resulting mixture was heated at 95°C in a closed reactor for two hours, forming a PHMB-MBDGA adduct. The adduct solution was cooled and filtered (Scientific Grade 417 filter). The resulting adduct solution contained 10% by weight of PHMB:BMDGA adduct having a PHMB:MBDGA ratio of 1.5:1.

(b) 330 mL of the 20% PHMB. This solution was combined with 100 ml of a sodium hydroxide (NaOH) solution containing 66 grams of NaOH, 66 ml of water and 34 ml of ethanol.

This mixture was added to a solution containing 40 grams of MBGDA, 120 ml of acetonitrile and 280 ml of ethanol. The resulting solution was heated at 95°C for 2 hours forming the PHMB-MBDGA adduct. The solution was cooled and filtered as described above. The resulting adduct solution contained 10% by weight of PHMB:MBDGA adduct having a PHMB:MBDGA ratio of 1.5:1.

The resins were characterized according to the following procedures:

1. Film formation was tested by a dip test with PE/PP (polyethylene/polypropylene) in which PE/PP samples were dipped in the resin solutions made in (a) and (b) above and dried by hot air blowing, and film formation was observed;
2. The ratio of polymer to crosslinker (PHMB-MBDGA) in the resin solution was tested by UV/visible spectroscopy;
3. Gelation time of the resin mixture was tested.

The resins were diluted with ethanol to a concentration of 1%. Film formation of the diluted resins were tested by the dip test with PE/PP as described above. Both resins formed a coherent film. The resins were stored in closed containers at ambient temperature.

Example 2

Coating of Plastic Articles

Various plastic articles were coated using the coating solutions described in Example 1.

1. contact lens cases: polyethylene and polypropylene contact lens cases were coated according to the following procedure:

The contact lens cases were cleaned by immersing them in absolute ethanol for 5 minutes and dried. The cleaned cases were immersed in the antimicrobial coating solution (Example 1a or 1b) for 1 to 2 minutes. The sample cases were dried by hot air blowing. Crosslinking was induced by heating the cases at 120°C for the polyethylene cases and at 200°C for the polypropylene cases for 2 hours. The cases

were allowed to cool, rinsed with 60°C water to remove any unbound polymer, then dried at 60°C for 1-3 hours.

The coated cases were immersed in a 0.05% solution of silver iodide/potassium iodide in alcohol for 2 minutes. The cases were rinsed with aqueous alcohol to remove any unbound silver. The cases then were rinsed with water and dried.

2. Toothbrush bristles: toothbrushes with nylon bristles were coated according to the procedure described for contact lens cases, except that the cross-linking reaction was carried out at 120-140°C.

3. Polyurethane and Polyvinylchloride Catheters: polyurethane and polyvinyl chloride catheters were coated according to the procedure described for contact lens cases, except that the crosslinking reaction was carried out at 80-120°C for polyurethane and at 120°C for polyvinylchloride.

4. Dental Water Line Unit Tubing And Filters: polyurethane tubing and polyethersulfone membrane and housing were coated according to the procedure described for contact lens cases, except that the crosslinking reaction was carried out at 80-120°C for polyurethane and 120-140°C for polyethersulfone.

5. Coating Process for Silicone Parts: The parts were pre-cleaned in 100% ethyl alcohol (reagent grade) to remove dirt, grease and other contaminants. They are then subjected to an alkaline etch by immersing them in a 0.1M NaOH in 90% ethanol solution (10% water) at room temperature and ultrasonicated for 2 minutes. They were then coated in an identical manner as the contact lens cases.

6. Coating Process for Teflon Parts: The parts were subjected to surface pretreatment by oxygen plasma for 5 minutes in a plasma reactor. They were then coated in an identical manner as the contact lens cases.

7. Coating Process for Nylon Sheets: Nylon sheets were pre-cleaned with 100% ethyl alcohol (reagent grade) to remove dirt, grease and other contaminants. The one

part formulation of coating resin has been diluted with 100% ethyl alcohol to the desired concentration of 1 wt.%. The cleaned nylon sheet was immersed in the coating resin for a period of 1-2 mins. Then, the sheet was carefully removed from the coating resin bath and the excess adhering resin was allowed to drain off. The coating on the nylon sheets was dried by placing them in an oven at 70°C for 3-4 mins. Then, dried resin coating was then crosslinked by thermal curing at 120°C for a period of 2 hours. The cured samples were removed from the oven and allowed to cool to room temperature.

This procedure was used to coat nylon toothbrush bristles, non-woven nylon and cellulose fibers.

Example 3

Membrane Coating Procedure

Polyethersulfone and nylon membranes were cleaned as described in Example 2 above. The membranes were coated with the antimicrobial resin solution described in Example 1 (1a or 1b) and dried. The coatings then were crosslinked by heating at 120°C for approximately 2 hours. The resulting crosslinked coatings were rinsed with water to remove any unbound polymer, were rinsed with acidified water or buffer [pH 2-2.5], followed by another water rinse, then dried. Silver was deposited into the crosslinked polymer matrix by immersing the coated membrane in a 0.05% solution of a silver iodide/potassium iodide complex in aqueous alcohol.

Unbound silver iodide was removed by an ethanol wash. The membrane was rinsed with water, then dried at 70°C for 30 minutes.

Example 4

Contact Killing Ability

The coated articles described in Example 2 and the membranes described in Example 3 were exposed to a variety of bacteria from the following genera: *Pseudomonas, Staphylococcus, Serratia, Klebsiella, Bacillus, Enterococcus* and

Aspergillus, and a fungus from the genus *Candida*. The species of microorganisms used are listed in Tables 1 and 2.

The articles and membranes were incubated with the microorganisms at 35-30°C for at least 20 hours, and for as long as 504 hours (21 days). The results are shown in Tables 1 and 2:

TABLE 1

Biocidal activity of treated surface

Organism	Challenge	Time to Kill Complete kill at 30°C
Pseudomonas dimunata	10^6CFU/mL	20 hours
Pseudomonas cepacia	10^6CFU/mL	20 hours
Staphylococcus aureus	10^6CFU/mL	20 hours
Serratia marcescens	10^6CFU/mL	20 hours
Escherichia coli	10^6CFU/mL	20 hours
Klebsiella pneumoniae	10^6CFU/mL	20 hours
Bacillus subtilis	10^6CFU/mL	20 hours
Bacillus cerius	10^6CFU/mL	20 hours
Staphylococcus epidermidis	10^5CFU/mL	72 hours
Enterococcus faecalis	10^6CFU/mL	20 hours
Candida albicans	10^6CFU/mL	168 hours
Aspergillus niger	10^5CFU/mL	no growth*

* 21 days at 25°C

TABLE 2

Bacterial Growthrough Challenge of Treated Membranes with 10^6 CFU/mL *Pseudomonas aeruginosa* In PBS at 30°C

Membrane Type	Days in Test	Days to Failure
Nylon Membrane, 0.2 µm, untreated control	32	30
Nylon Membrane, 0.2 µm, AMS coated	54	None
Polyether sulfone 0.2 µm, untreated control	5	3
Polyether sulfone 0.2 µm, AMS coated	70	None

AMS = antimicrobial surface

Example 5

Kinetics of antimicrobial action

The coating acts upon contact with the micro-organism, first intercalating into the cell membrane and second transferring the bio-toxic agent directly to the contacting organism. The following time to kill experiment was performed on polyethylene tubes with various diameters coated with the PHMB-MBDGA-silver coating described in Example 1. Coatings were applied as described in Example 2 for the contact lens cases. The tubes were inoculated with predetermined volumes of initial concentrations of up to 10^9 cfu/mL of *Pseudomonas aeruginosa* (ATCC#9027) in PBS and incubated at 30°C for 20 hours. At various time points tubes were sampled and the micro-organism was plated and counted. The treated tubes demonstrated significant antibacterial activity even when volume to surface ratios (S/V) exceeded 4:1. The results are shown in Figure 3.

Additional evidence for the contact killing mechanism was provided by the following experiment. Polypropylene tubes were coated as described in Example 2 for contact lens cases. The coated tubes and untreated controls were challenged with 10^6 cfu/ml of *Pseudomonas aeruginosa* in PBS at 30°C for 20 hours. An organism count by standard plating techniques showed no viable organisms, i.e., a complete elimination (6 log decrease) compared to the untreated tubes.

The solution containing the dead bacteria from the coated tubes was digested in 0.1M nitric acid and analyzed for the presence of silver. Silver concentration was found to be about 600 ppb. A coated tube containing blank PBS (no bacteria) incubated for the same time showed no detectable silver in the solution (less than 10 ppb).

Example 6

Non-leachability of the Coatings

To simulate an aging of approximately 1 year at ambient temperature, membranes with very large surface area were coated as described in Example 3. The coated membranes were immersed in water, isotonic saline and phosphate buffered saline solutions at 70°C for 5 days.

The test solutions were analyzed for elutables by spectroscopic methods with sensitivities less than 10 parts per billion (ppb) of active ingredients, i.e., PHMB, BMDGA, silver and iodide. The following levels were found:

Silver:	less than 10 ppb (below detection limit)
PHMB:	less than 100 ppb (below detection limit)
MBGDA:	less than 300 ppb (below quantitation limit)
Iodide:	less than 50 ppb (below quantitation limit)

These analytical results were further confirmed by testing the contact solutions to demonstrate that they show no antimicrobial activity by challenging them with silver sensitive *Escherichia coli* (ATCC # 8739) at a concentration of 10^6 cfu/mL. No decrease in numbers of the microorganism was detected after 20 hours.

Example 7

Toxicity

For assessing mammalian cell toxicity, polypropylene tubes coated as described in Example 2 for contact lens cases, were aged in phosphate buffered saline at 50°C for 48 hours. Test solutions were evaluated for toxicity with mouse fibroblast cells and showed no toxicity to the cells.

Example 8

Mechanical Strength

Treated surfaces coated as described in Example 2 were subjected to Sutherland rub test with 4 PSI for 50 strokes and remained viable while the rubbing surface did not show antimicrobial activity.

Example 9

Inertness

The coating remains fully inert and bio-active after exposure to a variety of physical and chemical stresses:

- Low temperature (-15°C), 24 hours
- Ethanol and boiling water, 1 hour
- Prolonged exposure to acidic and basic solutions of varying pH (4-10), 12 hours
- High ionic strength solution (2% sodium chloride), 24 hours
- Autoclaving (121°C for 15 minutes)
- Long term exposure to urine (35°C for 7 days)
- Challenged with 0.7% human serum albumin in phosphate buffered saline in accelerated aging tests (noted a small increase in non-bioavailable silver elutables due to protein complexation) at 80°C for 72 hours
- Exposure to blood products
- Worn by human volunteers for a 3 day period. No skin reaction was noted

Example 10

Surface Bio-Activity

The coating kills micro-organisms on contact - but is non-toxic to mammalian cells. In laboratory testing, treated surfaces (polypropylene, polyethylene, nylon and polyethersulfone) effectively eliminated all human pathogens tested - including bacteria, yeasts and fungi.

- *Bacillus cereus* (ATCC#11778)-10^6 cfu/mL in 20 hours
- *Escherichia coli* (ATCC#8739)-106 cfu/mL in 20 hours
- *Pseudomonas aeruginosa* (ATCC#9027)- 10^6 cfu/mL in 20 hours
- *Pseudomonas cepacia* (ATCC#25416)-10^5 cfu/mL in 20 hours
- *Pseudomonas diminuta* (ATCC#19146)- 10^6 cfu/mL in 20 hours
- *Klebsiella pneumoniae* (ATCC#13883)-10^6 cfu/mL in 20 hours
- *Staphyllococus aureus* (ATCC#6538)-10^6 cfu/mL in 20 hours

- *Serratia marcescens* (ATCC#8100)-106 cfu/mL in 20 hours
- *Enterococcus faecalis* (ATCC#19433)-10^6 cfu/mL in 20 hours
- *Staphyllococus epidermidis* (ATCC#12228)-10^5 cfu/mL in 72 hours
- *Candida albicans* (ATCC#10231)-10^5 cfu/mL in 168 hours

Surfaces coated as described in Example 2 were challenged with these microorganisms in the initial concentrations indicated. The microorganisms were suspended in phosphate buffered saline and were allowed to remain in contact with the treated surfaces for extended periods at 30°C. The solutions were then analyzed using standard plating methods. While organism growth was documented on untreated surfaces, the microorganisms were completely eliminated on the treated samples in the specified time period. These results were confirmed in thousands of tests conducted over three years.

In addition, the treated surfaces were tested against *Aspergillus niger*. No fungal growth was detected over the 28 day test period.

Example 11

Prevention of bio-film formation

To determine efficacy against bio-film formation, polyurethane tubes coated as described in Example 2 and untreated tubes were challenged with a mixture of the following micro-organisms, incubated in a 1% synthetic growth medium at room temperature.: *Pseudomonas Diminuta* (ATCC#19146), *Pseudomonas Aeruginosa* (ATCC#9027), *Klebsiella Pneumoniae* (ATCC#13883), *Bacillus cereus* (ATCC#11778), *Escherichia Coli* (ATCC#8739), *Staphylococcus aureus* (ATCC#6538). Within 24 hours, the micro-organisms in untreated tubes had grown from an initial concentration of 10^4 cfu/mL to an average of $3x10^5$ cfu/mL. The treated tubes had no viable micro-organisms.

The tubes were then washed and refilled with water. Eight days later, the untreated tubes still yielded 10^5 cfu/mL (resulting from the bio-film established during the first day of incubation) while the treated tubes yielded no micro-organisms.

Example 12

Antibiotic Resistant Bacteria

Untreated and treated (as described in Example 2) surfaces were challenged with 10^6 cfu/mL of methicillin and neomycin resistant strain of *Staphylococcus aureus* (ATCC#33592). The micro-organism was suspended in phosphate buffered saline and were allowed to remain in contact with the surfaces. Within 20 hours, treated surfaces had **no** viable organisms, whereas the number of viable organisms on untreated surfaces remained unchanged.

Example 13

Preparation of the Complex of Silver Iodide with Poly(hexamethylenebiguanide)

10 g of Cosmosil CQ (Zeneca, Biocides, Wilmington, DE), 10 ml of ethanol (EtOH) and 1.2 g of potassium iodide (KI) were mixed together. The resulting solution was added dropwise to 400 ml of an aqueous ethanol (1:1 v/v) solution containing 0.5% (w/v) of silver iodide and 6% (w/v) of potassium iodide. Precipitated white rubbery product was separated from the solution, rinsed with 50 ml of 50% (v/v) aqueous ethanol, and dried in a vacuum oven for 18 hours at 50°C. Silver containing product obtained after drying was a transparent light yellow color semisolid resin with a silver content of 10.7%.

Example 14

Preparation of Polyhexamethylenebiguanide base (PHMB) from Polyhexamethylenehydrochloride Solution

200 ml of Cosmocil CQ solution (Zeneca Biocides, Wilmington, DE) was neutralized by addition of 200 ml of aqueous NaOH (40 wt%) slowly with stirring, forming a precipitate. After filtering the supernatant liquid, the precipitate was suspended in 400 ml of alcohol. The PHMB suspension was diluted to 500 ml with additional alcohol and filtered. 10 ml of aliquot (filtered) gave 0.7 g of dried (100°C, 15 min) product. Calculated yield of PHMB solid in total solution was 35 g.

Example 15

Preparation of PHMB-Epoxide Resin (PHMB-MBDGA)

17.5 g of 4,4'-methylenebisdiglycidylaniline (MBDGA) (Aldrich Chemical Company, Milwaukee) was dissolved in 70 ml of acetonitrile. The resulting MBDGA solution was added in dropwise to the PHMB solution described in Example 14 that had been preheated in a water bath to 80-90°C with stirring. The reaction mixture turned turbid during the addition. The reaction was allowed to proceed for about 30 minutes at which time the solution became clear. The reaction vessel was removed from the bath and cooled. The pH of the cooled solution was adjusted to 3.65 by slow addition of 2N alcoholic HCl in alcohol.

Example 16

Preparation of the Complex of Silver Iodide with PHMB-MBDGA Resin

30 ml of 10% (w/v) ethanol solution of PHMB-MBDGA (2:1 w/w) resin (prepared as described in Example 15 above) was mixed with 30 ml of anhydrous ethanol and 3.6 g of potassium iodide. The resulting solution was added dropwise to 400 ml of an aqueous ethanol (1:1 v/v) solution containing 0.5% (w/v) of silver iodide and 6% (w/v) of potassium iodide. Precipitated white rubbery product was separated from the solution, rinsed with 50 ml of 50% (v/v) aqueous ethanol, and dried in vacuum oven for 18 hours at ambient temperature. The silver containing productobtained was a transparent light yellow color solid resin with a softening point 40-45°C and a silver content of 3.8%.

Example 17

Preparation of the Complex of Silver Iodide with Crosslinked PHMB-MBDGA Resin

500 ml of 10% (w/v) ethanol solution of PHMB-MBDGA (2:1 w/w) resin was prepared as in Examples 15-17 above. The volume of obtained solution was reduced to approximately 100 ml by solvent evaporation under vacuum in a rotovapor at a water bath temperature of 70°C. To accomplish solvent removal, the resulting viscous resin solution was transferred into glass beaker and placed in vacuum oven at ambient temperature. After 30 minutes of drying, the oven temperature was increased to 75°C and the sample was left in vacuum for another 16 hours. The

solid product obtained was cured at 130°C for 2 hours in a regular oven. The cured resin was ground to make resin powder with a particle size about 50 microns. A quantitative yield of crosslinked PHMB-MBDGA resin (>90%) was obtained at the end of the powder preparation process.

10 g of silver iodide and 55 g of potassium iodide were dissolved in a mixture of 50 ml of water and 150 ml of ethanol. Crosslinked resin powder (15 g) prepared as described above was immersed in the silver solution and left under agitation for 30 minutes. Then solid matter was separated from the supernatant and resuspended in 100 ml of anhydrous ethanol. After 10 minutes of washing, the resin powder was recovered from the mixture, rinsed with a fresh portion of alcohol (50 ml) and dried under vacuum at room temperature for 16 hours. 22 g of silver loaded crosslinked PHMB-MBDGA resin powder with a 5.9% silver content was obtained after complete solvent evacuation. Before use, the resin powder was ground and sieved through a standard testing sieve to get the particle size below 53 microns.

Example 18

The broad spectrum antimicrobial activity of the resin powder made as described in Examples 13-17 was evaluated as follows. The powder was suspended in phosphate buffered saline (PBS) at resin concentrations ranging from 0.05 mg/mL to 100 mg/mL.

The suspensions were inoculated with the following challenge microorganisms:

Pseudomonas aeruginosa	*ATCC 9027*
Escherichia coli	*ATCC 8739*
Staphylococcus aureus	*ATCC 6538*
Serratia marcescens	*ATCC 8100*
Staphylococcus epidermidis	*ATCC 12228*
Candida albicans	*ATCC 10231*

All microorganisms were inoculated as a suspension in PBS to the resin suspensions in PBS. PBS solutions without resin powder were used as controls. A bacterial challenge level of approximately 10^6 cfu/mL was maintained for all resin concentrations. After inoculation, the solutions were incubated at 30°C for 20 hours, following which the number of viable organisms

was determined by the standard spread plate method. A pass criteria was set for the antimicrobial efficacy of the resin suspensions which required the complete elimination of each type of microorganism over the 20 hour time period.

Test Results

Table 1. Antimicrobial Efficacy of Resin Powder Against Bacteria

Sample (mg/mL)	Organism	Final Conc.* (CFU/mL)	Sample (mg/mL)	Organism	Final Conc.* (CFU/mL)
Control	*P. aeruginosa*	$4.5x10^6$	Control	*S. marcescens*	$3.1x10^5$
100	*P. aeruginosa*	0	1	*S. marcescens*	0
10	*P. aeruginosa*	0	10	*S. marcescens*	0
1	*P. aeruginosa*	0	100	*S. marcescens*	0
Control	*E. coli*	$1.75x10^6$	Control	*S. epidermidis*	$3.85x10^5$
100	*E. coli*	0	1	*S. epidermidis*	0
10	*E. coli*	0	10	*S. epidermidis*	0
1	*E. coli*	0	100	*S. epidermidis*	0
Control	*S. aureus*	$2.4x10^6$			
100	*S. aureus*	0			
10	*S. aureus*	0			
1	*S. aureus*	0			

*Average value

Table 2. Antimicrobial Efficacy of Resin Powder Against Bacteria and Yeast

Sample (mg/mL)	Organism	Final Conc.* (CFU/mL)	Sample (mg/mL)	Organism	Final Conc.* (CFU/mL)
Control	P. aeruginosa	$9.3x10^4$	Control	S. marcescens	$1.75x10^6$
1.00	P. aeruginosa	0	1.00	S. marcescens	0
0.75	P. aeruginosa	0	0.75	S. marcescens	TCF/O*
0.50	P. aeruginosa	0	0.50	S. marcescens	0
0.25	P. aeruginosa	0	0.25	S. marcescens	TFC/0*
0.10	P. aeruginosa	0	0.10	S. marcescens	0
0.05	P. aeruginosa	0	0.05	S. marcescens	0
Control	E. coli	$8.2x10^4$	Control	C. albicans	$2.6x10^5$
1.00	E. coli	0	1.00	C. albicans	0
0.75	E. coli	0	0.75	C. albicans	0
0.50	E. coli	0	0.50	C. albicans	0
0.25	E. coli	0	0.25	C. albicans	TCF/O**
0.10	E. coli	0	0.10	C. albicans	0
0.05	E. coli	***	0.05	C. albicans	0
Control	S. aureus	$1.3x10^5$			
1.00	S. aureus	0			
0.75	S. aureus	0			
0.50	S. aureus	0			
0.25	S. aureus	0			
0.10	S. aureus	0			
0.05	S. aureus	0			

* Average value
**TCF/O - Number of colonies well below statistical relevance
*** Counts not available

The resin powder completely eliminated all microorganisms used in the study over the range of varying concentrations within a 20 hour time period over the entire range of resin concentrations.

Example 19

Preparation of Chain Extended PHMB by Reaction with Hydrophobic Epoxide

32.5 of Poly(Bisphenol A-coephichlorohydrin)glycidyl end capped (mol. wt. = 1075, Aldrich) was dissolved in 77 mL of N,N-dimethylformamide (DMF) with stirring. 250 mL of a 13% (by wt.) solution of PHMB (base) in 250 mL of absolute ethanol was added quickly. The turbid solution was heated in a water bath at 90°C for one hour with stirring. A clear viscous solution was obtained which was allowed to cool to room temperature that is immisicible with water.

Example 20

Preparation of Chain Extended PHMB by Reaction with Hydrophobic Epoxide

17.3 g of N,N-dyglycidylether-4-glycidyloxyaniline (Aldrich) was dissolved in 25 mL of N,N-dimethylformamide (DMF) with stirring. 130 mL of a 20% solution of PHMB.HCl (Cosmocil CQ, Zeneca Biocides, Delaware) was added quickly followed by 70 mL of distilled water. The turbid solution was refluxed in a water bath for 2 hours with stirring. A clear viscous solution was obtained which was allowed to cool to room temperature. The resulting chain extended compound is immisicible with water or pure ethanol, but is miscible in a solution of 50% aqueous ethanol.

What is claimed is:

CLAIMS

1. An antimicrobial composition comprising a stable, isolatable substantially water-insoluble complex of a polycationic material and a metallic material.

2. The composition of claim 1 comprising a complex of a polycationic polymeric material and a biocidal metallic material.

3. The composition of claim 1 wherein the polycationic organic material is a biguanide compound.

4. The composition of claim 1 wherein the biguanide compound is a polyhexamethylene biguanide, a salt thereof or a derivative thereof.

5. The composition claim 1 wherein said metallic material is selected from the group consisting of a metal, a metal oxide, a metal salt, a metal complex, a metal alloy, and combinations thereof.

6. The composition of claim 5 wherein the metal is silver.

7. The composition of claim 5 wherein the metallic material is silver iodide.

8. The composition of claim 1 which is in powder form.

9. A composition comprising an adduct of an antimicrobial polycationic material and one of

(i) a crosslinking agent containing at least three functional groups wherein said adduct is capable of forming a crosslinked network, or

(ii) a chain-extending agent comprising a substantially water insoluble mono-functional or difunctional organic compound;

wherein said adduct is substantially water insoluble.

10. The composition of claim 9 wherein the polycationic material is a biguanide compound.

11. The composition of claim 10 wherein the biguanide compound is a polyhexamethylene biguanide, a salt thereof or a derivative thereof.

12. The composition of claim 1 wherein the crosslinking agent is selected from the group consisting of multifunctional organic compounds containing functional groups including isocyanates, epoxides, carboxylic acids, acid chlorides, acid anhydrides, succimidyl ether aldehydes and ketones.

13. The composition of claim 9 wherein the chain-extending agent is selected from the group consisting of monofunctional or difunctional aliphatic hydrocarbons, heteroaliphatic hydrocarbons, aromatic hydrocarbons, heteroaromatic hydrocarbons, organosilanes and perfluoro compounds.

14. The composition of claim 1 further comprising a biocidal metallic material.

15. The composition of claim 14 wherein the biocidal metallic material is selected from the group consisting of a metal, a metal oxide, metal salt, a metal complex, a metal alloy and combinations thereof.

16. The composition of claim 15 wherein the metal is silver.

17. The composition of claim 15 wherein the metal salt is silver iodide.

18. A method for providing an antimicrobial surface for killing microorganisms on contact, comprising contacting a substrate with a composition comprising a substantially water-insoluble polycationic material and a metal salt thereby forming a layer on said substrate having a surface comprising a multiplicity of toxic metal salt reservoirs disposed within an organic matrix adhered to the substrate to form an adhered toxic metal salt/matrix complex which inhibits release of toxic metal into an aqueous solution in contact with the

surface but transfers toxic metal to proteins or glycoproteins of microorganisms contacted therewith in a concentration sufficient to kill the microorganisms.

19. The method of claim 18 wherein the metallic material is selected from the group consisting of a metal, a metal oxide, a metal salt, a metal complex, a metal alloy, and combinations thereof.

20. The method of claim 19 wherein the metal is silver.

21. The method of claim 20 wherein the metal salt is silver iodide.

22. The method of claim 18 wherein the polycationic material is a biguanide compound.

23. The method claim 22 wherein the biguanide compound comprises polyhexamethylene biguanide or derivatives thereof.

24. The method of claim 18 wherein the polycationic further comprises a multifunctional crosslinking agent or a monofunctional or difunctional chain-extending agent, wherein the polycationic material and the crosslinking agent or chain extending agent together form a substantially water-insoluble adduct.

25. The method of claim 24 wherein the crosslinking agent is selected from the group consisting of multifunctional compounds containing organic functional groups including isocyanates, epoxides, carboxylic acids, acid chlorides, acid anhydrides, succimidyl ethers aldehydes and ketones

26. The method of claim 24 wherein the polycationic material is an adduct of polyhexamethylene biguanide and a multifunctional crosslinking agent or a monofunctional or difunctional chain-extending agent.

27. A method of killing microorganisms on a surface of a substrate comprising the steps of:

providing a substrate having adhered thereto a polymeric matrix impregnated with toxic metal salt to form a toxic metal salt/matrix which inhibits leaching of the toxic metal from the adhered matrix into an ambient aqueous solution; and

inducing contact between the matrix and the microorganism to permit selective transfer of toxic metal into the microorganism in an amount sufficient to kill the microorganism.

28. The method of claim 27 wherein the toxic metal is silver.

29. The method of claim 28 wherein said metal salt is a silver halide.

30. The method of claim 27 wherein the polymeric matrix is a matrix formed from a biguanide polymer.

31. The method claim 30 wherein the polymer comprises polyhexamethylene biguanide or derivatives thereof.

32. The method of claim 27 wherein the polymer further comprises a multifunctional crosslinking agent or monofunctional or difunctional chain-extending agent, wherein the biguanide polymer and the crosslinking agent or chain extending agent together form an adduct, and wherein the adduct is substantially water-insoluble.

33. The method of claim 29 wherein the substrate is selected from the group consisting of metal, wood, natural and synthetic polymers, natural and synthetic fibers, cloth, paper, rubbers, and glass.

34. An article of manufacture comprising a medical device, health care device or personal care product having as a surface coating an antimicrobial composition comprising a complex of a polycationic material and a substantially water-insoluble biocidal metallic material.

35. The article of claim 34 comprising a medical device selected from the group consisting of catheters, urological devices, blood collection and transferring devices,

devices for implanting in a patient, urine collection devices, valves, stents, intraocular lenses, tracheotomy devices.

36. The article of claim 34 comprising a health care device selected from the group consisting of surgical gloves, surgical instruments, dental care instruments, dental consoles, dental unit water lines including tubing and filters contained within, instrument trays, ophthalmic devices, contact lenses, contact lens storage cases, topical disinfectants and wound dressings, storage containers, intravenous dispensers and syringes.

37. The article of claim 34 comprising a consumer product selected from the group consisting of hair care items, toothbrushes, dental floss, baby care items, child care items, bathroom accessories, bed linens, towels and wash cloths, water purification devices, kitchen implements, trash containers, disposable trash bags and cutting boards.

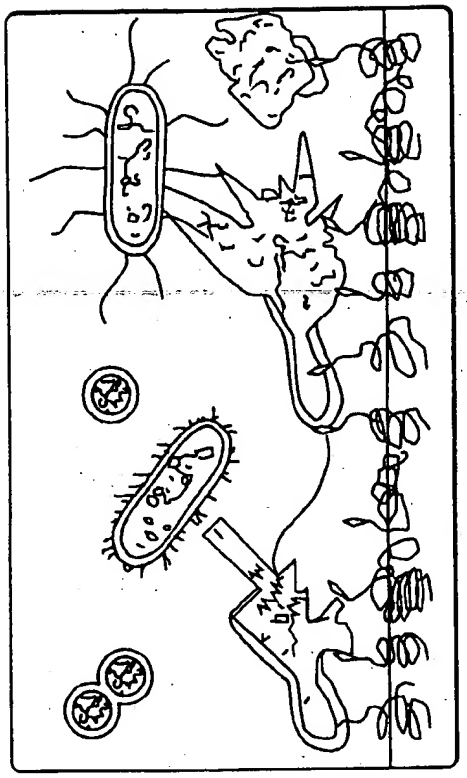


FIG. 1B

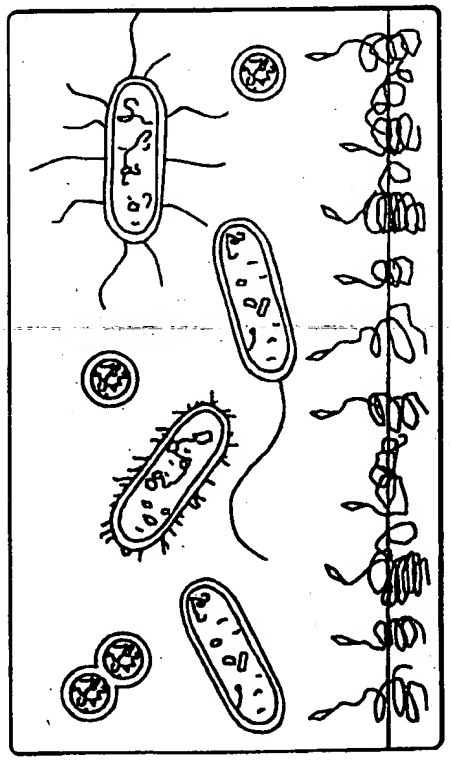


FIG. 1A

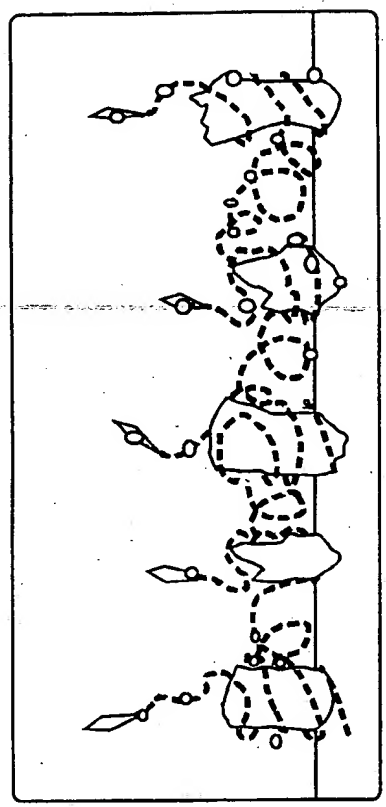


FIG. 2B

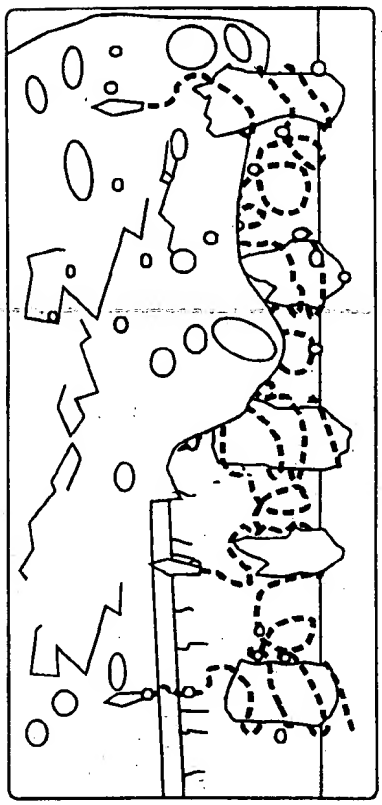


FIG. 2D

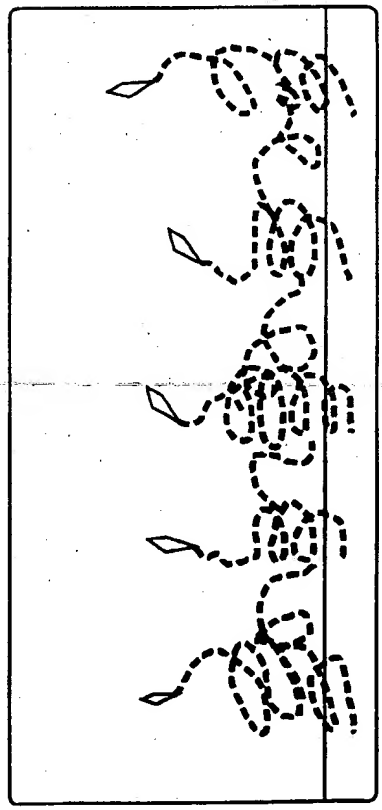


FIG. 2A

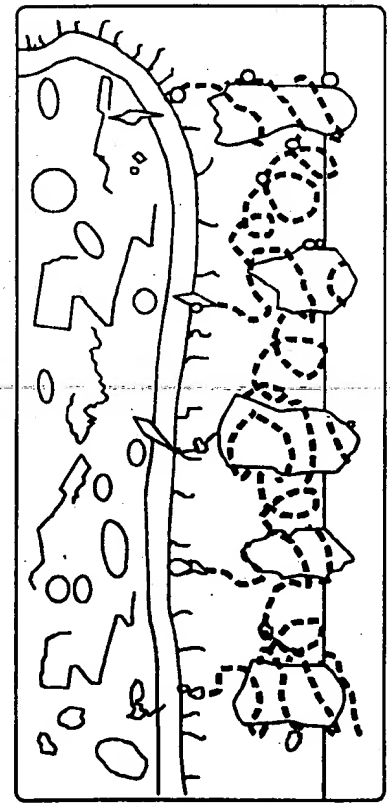
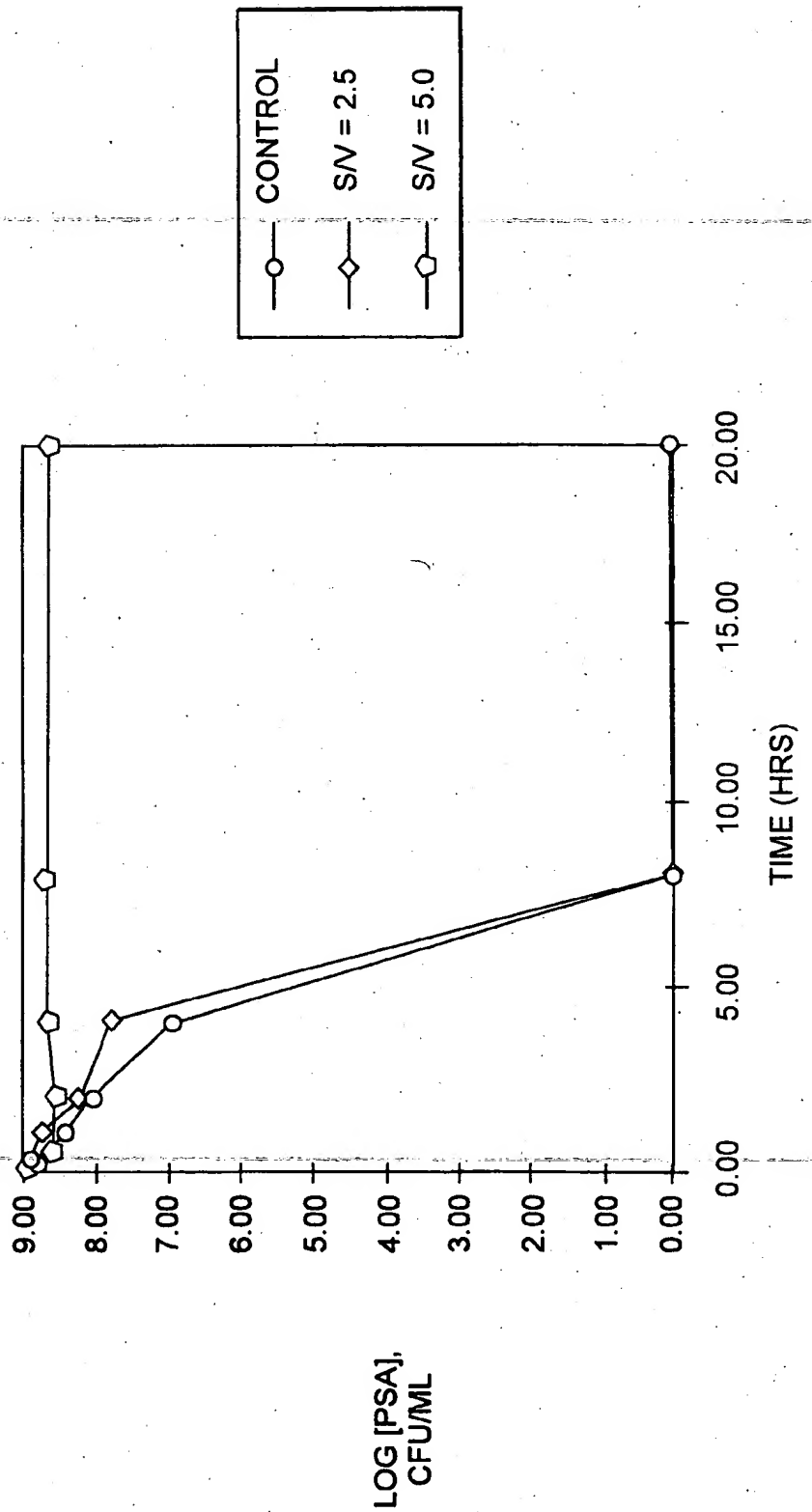


FIG. 2C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7.00
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5.00
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15.00
20.00
TIME (HRS)

FIG. 3

INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/US 97/19369

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A01N59/16

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A01N

Documentation searched other than minimumdocumentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 328 421 A (THE TRUSTTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK) 16 August 1989 see claims 24-35 see page 2, line 55 - page 3, line 13 see page 12, line 26 - line 55 ---	1-3,5-7, 18-22, 27-29, 33-37
X	WO 90 04390 A (THE TRUSTTES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK) 3 May 1990 see page 4, line 17 - line 28 see page 5, line 7 - line 17 see page 6, line 27 - page 7, line 2 see table 5B --- -/--	1-3,5-7, 18-22, 27-29, 33-37

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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1

Date of the actual completion of theinternational search	Date of mailing of the international search report
25 February 1998	06/03/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fort, M

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INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/US 97/19369

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

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Information on patent family members

Int tional Application No
PCT/US 97/19369

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